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Cytotoxic Constituents of the Octocoral *Dendronephthya gigantea*

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A known monoalkyl glycerol ether, (±)-1-nonadecyloxy-2,3-propanediol (**1**) was isolated from the ethyl acetate extract of a soft coral *Dendronephthya gigantea* as a weakly cytotoxic constituent against four human cancer cell lines, A549, HT-29, HT-1080, and SNU-638. In addition, a known ceramide, (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol (**2**), was also isolated as an inactive constituent. This is the first report on the isolation of the compounds **1** and **2** from the octocoral, *Dendronephthya* species.

Key words: Soft coral, *Dendronephthya gigantea*, (±)-1-Nonadecyloxy-2,3-propanediol, (2*S*, 3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol, Thymine, Uracil, Cytotoxicity

INTRODUCTION

The marine environment has been rich sources of bioactive substances including potent antitumor, anti-inflammatory, and analgesic compounds (Newman and Cragg, 2004). The octocoral, *Dendronephthya* species have been found to have interesting biological activities such as antimicrobial (Harder *et al.*, 2003) and antifouling effects (Tomono *et al.*, 1999a, 1999b; Miki *et al.*, 1996). Bioactive steroids (Tomono *et al.*, 1999a, 1999b), fatty acids (Mizobuchi *et al.*, 1993), alkaloids (Kawamata *et al.*, 1994), and glycosides (Rezanka and Dembitsky, 2003) have been reported previously from *Dendronephthya* species. There have not been many phytochemical and biological studies on *Dendronephhtya gigantea* which was exactly identified species, except for a report on the polyhydroxylated sterols with weak cytotoxic activity (Yoshikawa *et al.*, 2000).

As a part of our research program to find bioactive substances from natural resources, the octocoral, *D. gigantea* was collected in Jeju Island for further study. It was subjected to detailed laboratory investigation since its ethyl acetate extract exhibited considerable cytotoxic activity during preliminary screening. Bioassay-guided fractionation led to the isolation of a monoalkyl glycerol ether as cytotoxic constituents, along with a ceramide which is inactive. These compounds were identified by comparision with published values (Quijano *et al.*, 1994; Seo *et al.*, 1999; Shin and Seo, 1995) and with the aid of spectral analysis. To the best of our knowledge, the isolates, **1** and **2**, have never been reported previously from the soft coral, *Dendronephthya* species.

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MATERIALS AND METHODS

General experimental procedures

Melting points were measured on a J-923 melting point apparatus (Jisico, Korea) and are uncorrected. Optical rotation was measured with a P-1010 polarimeter (Jasco, Japan) at 25 °C. ^{1}H, ^{13}C, DEPT, COSY, HSQC, and HMBC NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA, USA). TMS was used as internal standard. EIMS was recorded on a JMS 700 GC-mass spectrometer (JEOL, Japan). Column chromatography was carried out on Si gel 60 (230-400 mesh, Merck, Germany) with mild nitrogen pressure for flash chromatography or Sephadex LH-20 (Amersham Pharmacia Biotech AB, Sweden). Fractions were monitored by TLC (Si gel 60 F_{254} plates, 0.25 mm thickness) with visualization under UV light (254 and 365 nm) and 10% sulfuric acid spray followed by heating.

Animal material

D. gigantea was collected in the sea of Jeju Island in March, 2003, and identified by one of the co-authors, Prof. Jun-Im Song (College of Natural Sciences, Ewha Womans University).

Extraction and isolation

The octocoral, *D. gigantea* (1.5 kg, wet weight) was ground and extracted with MeOH (1 L × 5) by percolation at room temperature. The filtered MeOH extract was evaporated under vacuum. The aqueous MeOH extract was partitioned with *n*-hexane, EtOAc, and *n*-BuOH, sequentially. The EtOAc extract (1.7 g) was subjected to a silica gel flash column chromatography using *n*-hexane-EtOAc-MeOH (gradient, 100:0:0 → 0:0:100) as a solvent system, to produce 18 fractions. Fraction 7 eluted with *n*-hexane-EtOAc (5:1) was further chromatographed using Sephadex LH-20 with 100% MeOH. Subfraction 3 from Sephadex LH-20 column chromatography afforded crude precipitate of compound **1**. Compound **1** (10 mg) was recrystallized from *n*-hexane. The crude precipitate of compound **2** obtained from fraction 9 eluted with *n*-hexane-EtOAc-MeOH (5:1:0.05) was recrystallized from MeOH to afford compound **2** (11 mg). Compound **3** (13 mg) was obtained from fraction 12 eluted with *n*-hexane-EtOAc-MeOH (5:1:0.1) from the first column chromatography. Fraction 14 eluted with *n*-hexane-EtOAc-MeOH (5:1:0.25) from the first column chromatography afforded crude precipitate of compound **4**. Compound **4** (6 mg) was recrystallized from EtOAc.

(±)-1-Nonadecyloxy-2,3-propanediol (1)

White powder; mp 49-51 °C; $[\alpha]_D^{25}$ 0.00° (MeOH, c 0.134); EIMS *m/z* (%) 359 (5) $[M+1]^+$, 327 (8), 313 (10), 267 (30), 57 (100); FABMS *m/z* 359.4 $[M+1]^+$; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 3.86 (1H, m, H-2), 3.73 (1H, dd, *J* = 11.4, 3.8 Hz, H-3), 3.65 (1H, dd, *J* = 11.4, 5.6 Hz, H-3), 3.54 (1H, dd, *J* = 9.6, 3.8 Hz, H-1), 3.50 (1H, dd, *J* = 9.6, 5.6 Hz, H-1), 3.46 (2H, dt, *J* = 6.7, 2.9 Hz, H-1'), 1.57 (2H, q, *J* = 6.8 Hz, H-2'), 1.26 (32H, br s, CH_2 × 16), 0.88 (3H, m, H-19'); ^{1}H-NMR (CD_3OD, 400 MHz) δ 3.75 (1H, m, H-2), 3.58 (1H, dd, *J* =11.2, 4.8 Hz, H-3), 3.50 (1H, dd, *J* = 11.2, 5.6 Hz, H-3), 3.48 (1H, m, H-1), 3.46 (2H, t, *J* = 6.8 Hz, H-1'), 3.41 (1H, dd, *J* = 10.0, 6.0 Hz, H-1), 1.57 (2H, m, H-2'), 1.29 (32H, br s, CH_2 × 16), 0.89 (3H, m, H-19'); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 72.7 (C-1), 72.1 (C-1'), 70.7 (C-2), 64.5 (C-3), 32.1 (C-17'), 29.9-29.6 (CH_2 × 14), 26.3 (C-2'), 22.9 (C-18'), 14.3 (C-19'); ^{13}C-NMR (CD_3OD, 100 MHz) δ 72.0 (C-1), 71.4 (C-1'), 71.1 (C-2), 63.4 (C-3), 31.8 (C-17'), 29.6-29.3 (CH_2 × 14), 26.0 (C-2'), 22.5 (C-18'), 13.2 (C-19'); ^{1}H-^{1}H COSY ($CDCl_3$, 400 MHz) H-2/H-3, H-1; H-2'/H-1', CH_2 × 16; ^{1}H-^{13}C HMBC ($CDCl_3$, 100 MHz): H-2/C-3; H-3/C-1, C-2; H-1/C-1', C-2, C-3; H-1'/C-1, C-2'; H-2'/C-1', CH_2 × 14; H-19'/ C-17', C-18'.

(2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol (2)

White powder; mp 87-89 °C; $[\alpha]_D^{25}$ -10.36° ($CHCl_3$, c 0.193); EIMS *m/z* (%) 535 (5) $[M]^+$, 298 (5), 281 (65), 250 (47), 239 (25), 85 (48), 60 (73); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 6.26 (1H, br d, *J* = 7.2 Hz, NH), 5.80 (1H, dt, 15.4, *J* = 6.4 Hz, H-5), 5.55 (1H, dt, *J* = 15.4, 6.4 Hz, H-4), 5.43 (1H, dt, *J* = 15.2, 6.4 Hz, H-9), 5.36 (1H, dt, *J* = 15.2, 6.4 Hz, H-8), 4.32 (1H, br t, *J* = 4.4 Hz, H-3), 3.95 (1H, dd, *J* = 11.0, 3.4 Hz, H-1), 3.91 (1H, m, H-2), 3.70 (1H, dd, *J* = 11.0, 3.4 Hz, H-1), 2.77 (2H, br s, OH), 2.23 (2H, t, *J* = 7.4 Hz, H-2'), 2.12 (2H, m, H-6), 2.08 (2H, m, H-7), 1.97 (2H, br dd, *J* = 13.2, 2.2 Hz, H-10), 1.64 (2H, br t, *J* = 7.4 Hz, H-3'), 1.28 (38H, br s, CH_2 × 19), 0.88 (6H, t, *J* = 6.8 Hz, H-18, H-16'); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.0 (C-1'), 133.5 (C-5), 131.4 (C-9), 129.2 (C-4), 127.0 (C-8), 74.7 (C-3), 62.5 (C-1), 54.4 (C-2), 36.8 (C-2'), 32.6 (C-10), 32.3 (C-6 or C-7), 32.1 (C-6 or C-7), 31.9 (CH_2 × 2), 29.7-29.2 (CH_2 × 15), 25.8 (C-3'), 22.7 (C-17, C-15'), 14.1 (C-18, C-16'); ^{1}H-^{1}H COSY ($CDCl_3$, 400 MHz) NH/H-2; H-2/H-3, H-1; H-4/H-3, H-5; H-5/H-6; H-8/H-9, H-7; H-9/H-10; H-2'/H-3'; CH_2 × 19/H-10, H-3', H-18, H-16'; ^{1}H-^{13}C HMBC ($CDCl_3$, 100 MHz) NH/C-1, C-2, C-1'; H-5/C-4, C-6; H-4/C-5, C-6; H-9/C-7, C-10; H-8/C-7, C-10; H-3/C-1, C-2; H-1/C-2, C-3; H-2/C-1; H-2'/C-1', C-3'; H-6/C-4, C-5; H-7/C-9; H-10/C-8; H-3'/ C-1'.

Thymine (3)

Pale yellow solid; mp 161 °C (dec.); EIMS *m/z* (%) 126 (100) $[M]^+$, 111 (15), 85 (20), 69 (30), 55 (90); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 10.99 (1H, br s, H-3), 10.59 (1H, br s, H-1), 7.25 (1H, d, *J* = 1.2 Hz, H-6), 1.73 (3H, d, *J* = 1.2 Hz, 5-Me); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 164.9 (C-4), 151.5 (C-2), 137.7 (C-6), 107.7 (C-5), 11.8 (5-Me); ^{1}H-^{13}C HMBC ($CDCl_3$, 100 MHz) H-Me/C-4, C-5, C-6; H-6/C-2, C-4, C-Me.

Uracil (4)

White solid; mp > 300 °C; EIMS *m/z* (%) 112 (100) $[M]^+$, 97 (9), 83 (10), 69 (52), 68 (13); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 10.99 (1H, br s, H-3), 10.92 (2H, br s, H-1 and H-3), 7.39 (1H, d, *J* = 7.2 Hz, H-6), 5.45 (1H, d, *J* = 8.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 163.9 (C-4), 151.1 (C-2), 141.8 (C-6), 99.8 (C-5).

Chemicals

All chemicals and reagents used were of highest purity. Trichloroacetic acid (TCA) and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Minimal essential medium with Earles salt (MEME), fetal bovine serum (FBS), non-essential amino acid solution (10 mM, 100X), trypsin-EDTA solution (1X) and antibiotic-antimycotic solution (PSF) were from GIBCO-BRL (Grand Island, NY, USA).

In vitro cytotoxicity assay

Cytotoxic potential was determined as described

previously (Lee *et al.*, 1998). Briefly, cells (in log growth phase) were counted, diluted to 5×10^4 cells/mL with fresh medium, and added to 96-well microtiter plates (190 μL/well) containing test materials (10 μL in 10% aqueous DMSO). Test plates were incubated for 3 days at 37 °C in CO_2 incubator. For zero day controls, cells were incubated for 30 min at 37 °C in CO_2 incubator. All treatments were performed in triplicate. After the incubation periods, cells were fixed by the addition of 50 μL of cold 50% aqueous trichloroacetic acid (4 °C for 30 min), washed 4-5 times with tap water, and air-dried. The fixed cells were stained with sulforhodamine B (SRB) (0.4% w/v SRB in 1% aqueous acetic acid) for 30 min. Free SRB solution was then removed by rinsing with 1% acetic acid. The plates were then air-dried, the bound dye was solubilized with 200 μL of 10 mM tris-base (pH 10.0), and absorbance was determined at 515 nm using an ELISA plate reader. Finally, the absorbance values obtained with each of the treatment procedures were averaged, and the averaged value obtained with the zero day control was subtracted. These results were expressed as a percentage, relative to solvent-treated control incubations, and IC_{50} values were calculated using non-linear regression analyses (percent survival versus concentration).

RESULTS AND DISCUSSION

The two known compounds, (±)-1-nonadecyloxy-2,3-propanediol (**1**) and (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol (**2**) were isolated from the ethyl acetate extract of *D. gigantea* by bioassay-guided fractionation using the cytotoxicity assay against several human cancer cell lines. Structures of **1** and **2** were identified by analysis of their NMR data as well as by comparison of their physical and spectral data with those of literature values (Quijano *et al.*, 1994; Seo *et al.*, 1999; Shin and Seo, 1995). To the best of our knowledge, compounds **1** and **2** have not been isolated from the *Dendronephthya* species. In addition, two primary metabolites, thymine (**3**, Ouwerkerk *et al.*, 2002) and uracil (**4**, Ouwerkerk *et al.*, 2002), were also isolated from this animal.

Compound **1** showed a protonated molecular ion peak $[M+1]^+$ at *m/z* 359 and other significant peaks at *m/z* 327 $[M-CH_3O]^+$, 313 $[M-C_2H_5O]^+$, and 267 $[M-C_3H_7O_3]^+$ in its LREIMS, indicating the presence of glycerol ether moiety and C_{19} saturated aliphatic chain in the molecule. The $[M+1]^+$ ion peak is a general phenomenon in the EI-mass spectrometry of a number of compounds such as alcohols, ethers, amines, glycols, and nitriles (McLafferty, 1957). The positive FAB mass spectrum of **1** also showed the $[M+1]^+$ ion peak at *m/z* 359, providing further evidence for the molecular formula. In the ^1H- and ^{13}C-NMR spectra, characteristic signals for the glycerol ether appeared at δ_H 3.86/δ_C 70.7, 3.73 and 3.65/ 64.5, 3.54 and 3.50/72.7, and 3.46/72.1. The HMBC correlations of H-1/C-1', C-2, and C-3 and H-1'/C-1 and C-2' provided the evidences that the C_{19} saturated aliphatic chain is connected to the glycerol group through an ether bridge. Thus compound **1** was identified as 1-nonadecyloxy-2,3-propanediol. It was confirmed by comparison of its ^1H- and ^{13}C-NMR data with published values (Quijano *et al.*, 1994; Seo *et al.*, 1999). Compound **1** was not optically active, indicating that it was a racemic mixture. Therefore, the chiral center at C-2 in **1** could not be determined.

Compound **2** gave the molecular ion peak at *m/z* 535 and showed the odd nitrogen number in its LREIMS. In the ^1H- and ^{13}C-NMR spectra, characteristic signals for an amide group appeared at δ_H 6.26 and δ_C 174.0. The ^1H- and ^{13}C-NMR spectra also showed signals for two olefinic groups at δ_H 5.80/δ_C 133.5, 5.55/129.2, 5.43/131.4, and 5.36/127.0. A strong signal at δ_H 1.28 (38H, br s) in the ^1H-NMR spectrum and the negative peaks (aliphatic methylenes) at δ_C 36.8~22.7 in the DEPT-135 NMR spectrum indicated the fatty acid moiety, which was confirmed by the COSY correlations of H-3'/H-2' and $C\underline{H}_2 \times 19$ and H-16'/$C\underline{H}_2 \times 19$. The COSY correlations of N<u>H</u>/H-2, H-2/H-1 and H-3, H-4/H-3 and H-5, H-5/H-6, H-8/H-7 and H-9, H-10/H-9 and $C\underline{H}_2 \times 19$, and $C\underline{H}_2 \times 19$/H-18 indicated the long chain base. The lengths of the long chain base and the fatty acid were determined as 2-amino-4,8-octadecadiene-1,3-diol and hexadecanoic acid, respectively, by EIMS, which showed significant fragment ion peaks at *m/z* 298 and 281. The HMBC correlations of N<u>H</u>/C-1', C-1, and C-2 and H-2'/C-1' and C-3' suggested that the fatty acid is connected to the long chin base through the amide group. The large coupling between the olefinic protons ($J_{4,5}$ = 15.4 Hz, $J_{8,9}$ = 15.2 Hz) provided

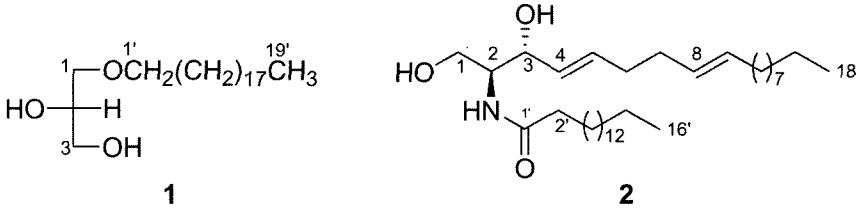

Fig. 1. Structures of isolates **1** and **2** from *D. gigantea*

that both of the double bonds have *E*-configuration. The absolute configurations at C-2 and C-3 of **2** were determined as 2*S* and 3*R*, respectively, by comparison of its optical rotation value ($[\alpha]_D^{25}$ -10.36°) and literature value ($[\alpha]_D^{25}$ -8.0°). Therefore, compound **2** was identified as the known compound, (2*S*,3*R*,4*E*,8*E*)-*N*-hexadecanoyl-2-amino-4,8-octadecadiene-1,3-diol. It was confirmed by comparison of its physical and spectral data with published values (Shin and Seo, 1995).

Cytotoxic activities of compounds **1** and **2** were evaluated against a panel of human cancer cell lines including lung carcinoma (A549), colorectal adenocarcinoma (HT-29), fibrosarcoma (HT-1080), and stomach adenocarcinoma (SNU-638). Compound **1** showed weak cytotoxic activity with IC_{50} values of 15.1, 14.5, 13.7, and 15.5 μg/mL against A549, HT-20, HT-1080, and SNU-638, respectively, which are comparable to the positive control, ellipticine (IC_{50} = 0.2~0.4 μg/mL). Compound **2** did not show any activity in the present study. To the best of our knowledge, this is the first report for the cytotoxic activity of compound **1**.

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